Remarks

The July 8, 2004 Official Action has been carefully reviewed. In view of the amendments submitted herewith and the following remarks, favorable reconsideration and allowance of this application are respectfully requested.

At the outset it is noted that a shortened statutory response period of three (3) months was set forth in the July 8, 2004 Official Action. Therefore, the initial due date for response was October 8, 2003. A petition for a one month extension of the response period is presented with this response, which is being filed within the one month extension period.

At page 2 of the Official Action, the Examiner has objected to the specification for having an improper heading. Applicant has addressed the Examiner's objection by changing the heading from "Brief Descriptions of the Drawing" to "Brief Description of the Drawings."

The Examiner has rejected claims 1-17 under 35 U.S.C. §112, second paragraph for alleged indefiniteness.

Claims 1-17 have been rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Dox et al. (J. Biol. Chem. (1922) 54:671-673) in view of Nudelman et al. (Synthetic Communications (1998) 28:471-474).

The foregoing objection and rejections constitute all of the grounds set forth in the July 8, 2004 Official Action for refusing the present application.

No new matter has been introduced into this application by reason of any of the amendments presented herewith. Moreover, none of the present claim amendments is believed to constitute a surrender of any originally claimed subject matter, or a narrowing of the claims in order to establish patentability. The effect of these amendments is merely to make explicit that which was implicit in the claims as originally worded.

CLAIMS 1-17, AS AMENDED, MEET THE REQUIREMENTS UNDER 35 U.S.C. §112, SECOND PARAGRAPH

The Examiner has rejected claims 1-17 and additionally rejected claims 3-9 under 35 U.S.C. §112, second paragraph for alleged indefiniteness for the following two reasons.

First, it is the Examiner's position that claims 1-17 are indefinite because it is unclear whether the temperature range provided in claim 1 refers to the reaction medium, to the generation of the acid catalyst, or both. Applicant has amended claim 1 to specifically recite that the reaction medium is heated to a temperature between about 35°C and about 50°C.

Second, the Examiner contends that claims 3-9 are indefinite because it is allegedly unclear whether claim 3 encompasses single compounds or compositions containing an alcohol and acyl halide. Applicant has deliberately used the term "comprises" in the context of claim 3 to encompass various commercial grades of ethanol and acetyl chloride which may be used in the practice of the invention. As is well known, the purity of chemical reagents, such as ethanol and acetyl chloride, varies according to grade. The possibility that there may be impurities in the ethanol or acetyl chloride, or both, does not make claims 1-17 indefinite. Ex parte Schuppner, 170 U.S.P.Q. 289 (Bd. Apps. 1970).

In light of the foregoing, Applicant submits that the rejection of claims 1-17 and the rejection of claims 3-9 for alleged indefiniteness are untenable. Accordingly, Applicant respectfully requests the rejections under 35 U.S.C. §112, second paragraph be withdrawn.

CLAIMS 1-17 ARE NOT RENDERED OBVIOUS BY DOX ET AL. IN VIEW OF NUDELMAN ET. AL.

The Examiner has rejected claims 1-17 under 35 U.S.C. §103(a) as allegedly unpatentable over Dox et al. in view of Nudelman et al. The Examiner contends that Dox et al. teach a process for the formation of creatine ethyl ester hydrochloride by saturating creatine in absolute ethanol with dry HCl gas. Dox et al. do not teach the *in situ* production of an acid catalyst. However, according to the Examiner, Nudelman et al. teach a method for performing esterifications in which HCl is produced *in situ* by the addition of acetyl chloride to an alcohol solution. It is the Examiner's position that a skilled artisan would have been motivated to combine the methods of Dox et al. and Nudelman et al. in order to arrive at the instantly claimed invention because of the desire to avoid the corrosive effects of the HCl gas employed by Dox et al.

Applicant respectfully disagrees with the Examiner. It is a well-settled principle of patent law that all claim recitations must be considered in determining non-obviousness under 35 U.S.C. §103. In re Saether, 181 U.S.P.Q. 36 (CCPA 1973). It has long been held that when the Examiner disregards specific claim recitations that distinguish over the prior art, the rejection is improper and will be over-turned. In re Glass, 176 U.S.P.Q. 489 (CCPA 1973). The Examiner contends that, in the method taught by Dox et al., the saturation of the creatine in absolute ethanol with HCl gas is performed "presumably with heating due to dissolution of HCl." The Examiner also interprets Nudelman et al. as teaching that the reaction mixture is heated by the exothermic reaction of generating the acid catalyst *in situ*. Applicant submits, however, that Dox et al. clearly teach that the HCl gas was added to the solution of creatine in alcohol until it was "saturated at room temperature" (page 672, lines 1-3).

Furthermore, Nudelman et al. also teach that ice cold solutions are employed initially to counteract the exothermic nature of the reaction and that the esterification reactions are "allowed to warm to room temperature" (page 474, lines 11-17). Accordingly, Applicant submits that Dox et al. and Nudelman et al. fail to teach the temperature recitation of applicant's claims or suggest any advantage to performing the reaction at a temperature above room temperature.

Furthermore, another criterion for determining obviousness under §103 is whether the prior art supplies some motivation or incentive to one of ordinary skill in the art to arrive at the invention as claimed. In re Dow Chemical Company, 5 U.S.P.Q. 2d 1929 (Fed. Cir. 1988). Applicant submits that a artisan of ordinary skill, based on information generally known in the art, would have been dissuaded from attempting the esterification of creatine at temperatures above room temperature because of a concern for creating creatinine. Indeed, it is generally known in the art that cyclization of creatine to creatinine can occur *in vitro* by nonenzymatic means. It is also known that elevated temperatures and low pH favors the formation of creatinine (see, for example, Wyss and Kaddurah-Daouk (Physiological Reviews (2000) 80:1107-1213) at page 1113, right column). Inasmuch as creatinine HCl is a dead-end product and cannot be converted to the desired product creatine ester HCl (see page 9, lines 14-22 of the instant specification and Mold, J.D. et al. (J. Amer. Chem. Soc. (1955) 77:178-180) at page 178, right column), a skilled artisan would not have the requisite motivation or reasonable expectation of success for increasing the reaction temperature above room temperature.

The MPEP at §716.02 clearly indicates that the demonstration of greater than expected results are evidence of nonobviousness. Indeed, "a greater than expected result is an evidentiary factor pertinent to the legal conclusion of

obviousness … of the claims at issue." In re Corkill, 226 U.S.P.Q. 1005 (Fed. Cir. 1985). At page 11, lines 27-30 of the instant specification, Applicant discloses that a method similar to the method of Dox et al., wherein HCl gas is employed instead of the *in situ* generation of an acid catalyst, resulted in a yield of only 48%-63%. However, the instantly claimed method resulted in a yield of 74% creatine ethyl ester HCl (page 11, lines 21-25). Inasmuch as Nudelman et al. is silent as to any effect the *in situ* production of the acid catalyst would have on yield, the instant results must be considered unexpectedly superior.

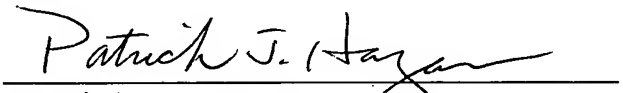
Therefore, Applicant submits that claims 1-17 cannot be considered to be obvious over Dox et al. in view of Nudelman et al. because of 1) the failure of the prior art to teach or suggest that elevated reaction temperatures would give rise to substantial production of creatine esters, and 2) the unexpectedly superior yield of creatine ester obtained from employing *in situ* generated acid compared to employing HCl gas. Accordingly, Applicant respectfully requests the withdrawal of the rejection of claims 1-17 under 35 U.S.C. §103(a).

CONCLUSION

In view of the amendments presented herewith, and the foregoing remarks, it is respectfully urged that the objection and rejections set forth in the July 8, 2004 Official Action be withdrawn and that this application be passed to issue.

In the event the Examiner is not persuaded as to the allowability of any claim, and it appears that any outstanding issues may be resolved through a telephone interview, the Examiner is requested to telephone the undersigned attorney at the phone number give below.

Respectfully submitted,
DANN, DORFMAN, HERRELL AND SKILLMAN
A Professional Corporation

By 

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Creatine and Creatinine Metabolism

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Wyss, Markus, and Rima Kaddurah-Daouk. Creatine and Creatinine Metabolism. *Physiol Rev* 80: 1107–1213, 2000.—The goal of this review is to present a comprehensive survey of the many intriguing facets of creatine (Cr) and creatinine metabolism, encompassing the pathways and regulation of Cr biosynthesis and degradation, species and tissue distribution of the enzymes and metabolites involved, and of the inherent implications for physiology and human pathology. Very recently, a series of new discoveries have been made that are bound to have distinguished implications for bioenergetics, physiology, human pathology, and clinical diagnosis and that suggest that deregula-

(Cr + PCr; Fig. 5). There may be only two exceptions. *1*) Kidney displays a much higher Cr transporter content than expected from its CK activity (Fig. 5*A*, ■), which might be due to an involvement of the Cr transporter in the resorption of Cr from the primary urine. *2*) Liver has a considerably lower CK activity than expected from its Cr content (Fig. 5*B*, ▲), which may be an expression of a strict separation between Cr-synthesizing and CK-expressing tissues in the body. Such a separation may be a crucial prerequisite for independent regulation of Cr biosynthesis on one hand and CK function/energy metabolism on the other hand.

Resting type 2a and 2b skeletal muscle fibers of rodents contain ~32 mM PCr and 7 mM Cr, whereas type 1 fibers comprise ~16 mM PCr and 7 mM Cr (525). The difference in PCr concentration between type 1 and type 2 muscle fibers is less pronounced in humans (337, 844, 1042); nevertheless, the concentration of total Cr seems to parallel the muscle glycolytic capacity in both rodents and humans. In serum and erythrocytes, as opposite extremes, [Cr] amounts to only 25–100 μM and 270–400 μM, respectively (175, 776, 1137), implying that Cr has to be accumulated by most Cr-containing tissues against a large concentration gradient from the blood. This accumulation via the Cr transporter is driven by the electrochemical potential differences of extracellular versus intracellular [Na^+] and [Cl^-].

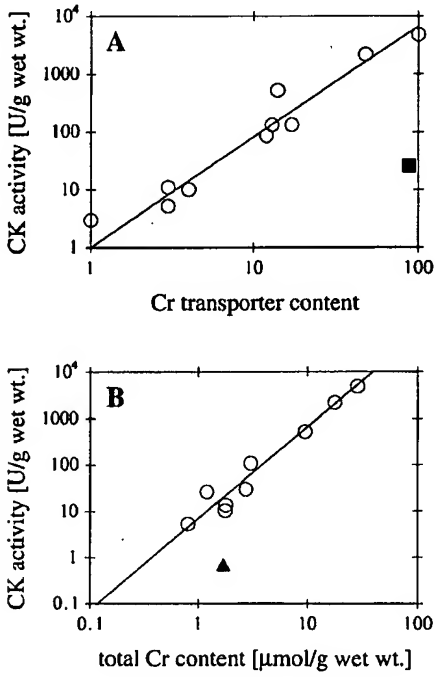

FIG. 5. Correlations between Cr transporter level, CK activity, and total Cr content in different mammalian (rat, human, cat, dog, rabbit, mouse, and guinea pig) tissues. The respective tissues are, from *left* to *right*, as follows: *A*: pancreas, spleen, ovary, lung, small intestine, prostate, brain, colon, heart, kidney (■), and skeletal muscle. *B*: spleen, kidney, liver (▲), smooth muscle (carotid artery), macrophages, brown adipose tissue, uterus, brain, heart, and skeletal muscle. (Data were taken from Refs. 56, 61, 129, 137, 172, 227, 529, 569, 570, 691, 1020, 1030.)

Because both seminal vesicles and seminal vesicle fluid of the rat and mouse contain considerable quantities of Cr and PCr, it was hypothesized "that both compounds are actively secreted by the seminal vesicle epithelium" (542). This hypothesis later turned out to be incorrect, in as far as seminal vesicles were shown to lack AGAT and GAMT but to contain moderate amounts of Cr transporter mRNA (543). Therefore, seminal vesicles most likely accumulate Cr from the blood.

For PCr and Cr, a single cytosolic pool is assumed by most researchers, especially by those who postulate near-equilibrium conditions for the CK reaction throughout the cell. However, tracer studies with [^{14}C]Cr suggested distinct cytosolic pools of Cr in rat heart (850) and fast-twitch (white) muscle of the rainbow trout (369). In addition, quantitative X-ray microanalysis revealed that phosphorus compounds (presumably represented mostly by PCr and ATP) are highly compartmentalized in sarcomeric muscle, with a preferential occupancy of the I band as well as the H zone (549). Surprisingly, some researchers detected Cr and PCr in the matrix of heart mitochondria and provided evidence that PCr uptake into the mitochondrial matrix is mediated by the adenine nucleotide translocase (see Refs. 391, 796, 921). In the light of *1*) the lower phosphorylation potential within the mitochondrial matrix compared with the cytosol and *2*) the lack of Cr-utilizing processes in the mitochondrial matrix, it is questionable whether and to what extent Cr accumulation in the matrix is physiologically relevant or is due to postmortem or other artifacts. Clearly, further studies are needed to get a deeper insight into the subcellular compartmentation of Cr and PCr.

C. Degradation of Cr and PCr in Vertebrates

The degradation of Cr and PCr in vertebrates is, for the most part, a spontaneous, nonenzymatic process, as indicated in the top part of Figure 2. In vitro, the equilibrium of the reversible and nonenzymatic cyclization of Cr to creatinine (Cr ↔ Crn) is both pH dependent and temperature dependent. Cr is favored at high pH and low temperature, whereas Crn (M_r 113.1) is favored at elevated temperatures and in acidic solutions (see Ref. 551). In both directions, the reaction is monomolecular. Starting with pure Cr solutions, 1.0–1.3% of the Cr per day is converted into Crn at pH 7.0–7.2 and 38°C. In vitro studies on the stability of PCr revealed that this high-energy phosphate compound is acid labile, yielding P_i and either Cr or Crn upon hydrolysis. Both the rate of PCr hydrolysis and the ratio of Cr to Crn formed depend on temperature and pH and can additionally be influenced in a concen-

tration-dependent manner by molybdate (for reviews, see Refs. 226, 669).

In contrast to these in vitro studies, experiments with ^{15}N-labeled compounds clearly showed that the conversion of Cr into Crn in vivo is an irreversible process (72). Upon feeding of rats with [^{15}N]Cr, the isotopically labeled Cr distributed homogeneously over the total Cr pool in the body as well as over the urinary Crn. Even after 5 days, the specific labeling of the urinary Crn and the body Cr were still the same, suggesting that Cr is the only precursor of Crn. Upon feeding with [^{15}N]Crn, however, most of the label was directly excreted into the urine, and no significant exchange of the label with the body Cr was observed. In accordance with in vitro studies, an almost constant fraction of the body Cr (1.1%/day) and PCr (2.6%/day) is converted nonenzymatically into Crn in vivo, giving an overall conversion rate for the total Cr pool (Cr + PCr) of ~1.7%/day (for a review, see Ref. 1077). Consequently, in a 70-kg man containing ~120 g of total Cr, roughly 2 g/day are converted into Crn and have to be replaced by Cr from the diet or from de novo biosynthesis (Fig. 4) (1050, 1077, 1085). With the assumption of an average content in muscle of 30 mM of total Cr (see above) and a quantitative uptake of the compound by the digestive tract, this loss could be compensated by ingestion of 500 g of raw meat per day. Because Crn is a very poor substrate of the Cr transporter (318, 319, 691), because no other specific saturable uptake mechanism exists for Crn (515), and because Crn, most likely due to its nonionic nature, is membrane permeable, Crn constantly diffuses out of the tissues into the blood and is excreted by the kidneys into the urine (Fig. 4) (759). Because the rate of nonenzymatic formation of Crn from Cr is nearly constant, and because >90% of the total bodily Cr is to be found in muscle tissue, 24-h urinary Crn excretion is frequently used as a rough measure of total muscle mass (768, 1067). However, this approach suffers various limitations.

Twenty to twenty-five percent of the in vivo conversion of PCr into Crn may proceed via phosphorylcreatinine (PCrn) as an intermediate (414). Accordingly, [PCrn] in rabbit white skeletal muscle was found to be 0.4% of [PCr], and commercial preparations of PCr (at least several years ago) contained 0.3–0.7% of PCrn.

Crn in mammals, and especially in humans, is still widely believed to be an inert substance that is excreted as such into the urine. Several lines of evidence, however, contradict this view. Using radiolabeled Crn, Boroujerdi and Mattocks (83) showed that in rabbits, some Crn is converted into Cr, Arg, guanidinobutyrate, or guanidinopropionate. Additional routes of Crn degradation become favored in states of renal insufficiency and seem to be relevant for human pathology. They are therefore discussed in detail in section IX*H*.

V. REGULATION OF CREATINE METABOLISM IN VERTEBRATES

In keeping with the rather complex organization of Cr biosynthesis and degradation in vertebrates, a variety of potential regulatory mechanisms have to be considered, for instance, allosteric regulation, covalent modification, or changes in expression levels of the enzymes involved in Cr metabolism. In addition, changes in the transport capacity and/or permeability of biological membranes for the intermediary metabolites, i.e., Cr, Crn, and guanidinoacetate, are also expected to have an impact on Cr metabolism as a whole (for an extensive review, see Ref. 1077).

A. Regulation of L-Arginine:glycine Amidinotransferase Expression and Activity

The formation of guanidinoacetate is normally the rate-limiting step of Cr biosynthesis (see Ref. 1077). Consequently, the AGAT reaction is the most likely control step in the pathway, a hypothesis that is supported by a great deal of experimental work. Most important in this respect is the feedback repression of AGAT by Cr, the end-product of the pathway, which most probably serves to conserve the dietary essential amino acids Arg and Met. Circumstantial evidence indicates that in folic acid deficiency, where Cr biosynthesis is curtailed and the serum concentration of Cr is likely to be decreased, AGAT expression is upregulated (187). In contrast, an increase in the serum concentration of Cr, due either to an endogenous source or to dietary Cr supplementation, results in concomitant decreases in the mRNA content, the enzyme level, and the enzymatic activity of AGAT, thus suggesting regulation of AGAT expression at a pretranslational level (322, 1053; for a review, see Ref. 1077). Feedback repression of AGAT by Cr is most pronounced in kidney and pancreas, the main tissues of guanidinoacetate formation, but is also observed in the decidua of pregnant rats (see Ref. 1077). Immunological studies suggest the presence of multiple forms (or isoenzymes) of AGAT in rat kidney, of which only some are repressible by Cr, whereas others are not (314). Because the half-life of AGAT in rat kidney is 2–3 days (624), the changes in the AGAT levels described here are rather slow processes, thus only allowing for long-term adaptations.

Cyclocreatine, *N*-acetimidoylsarcosine, and *N*-ethylguanidinoacetate display repressor activity like Cr, whereas Crn, PCr, *N*-propylguanidinoacetate, *N*-methyl-3-guanidinopropionate, *N*-acetimidoylglycine, and a variety of other compounds are ineffective (809, 1077). L-Arg and guanidinoacetate have only "apparent" repressor activity. They exert no effect on AGAT expression by themselves but are readily converted to Cr, which then acts as the true repressor.